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Import of assembled PTS1 proteins into peroxisomes of the yeast *Hansenula polymorpha*: Yes and No!

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Abstract

Previously, Waterham et al. [EMBO J. 12 (1993) 4785] reported that cytosolic oligomeric alcohol oxidase (AO) is not incorporated into peroxisomes after reassembly of the organelles in the temperature-sensitive peroxisome-deficient mutant *pex1-6^{ts}* of *Hansenula polymorpha* shifted to permissive growth conditions. Here, we show that the failure to import assembled AO protein is not exemplary for other folded proteins because both an artificial peroxisomal matrix protein, PTS1-tagged GFP (GFP.SKL), and the endogenous dimeric PTS1 protein dihydroxyacetone synthase (DHAS) were imported under identical conditions. In vitro receptor–ligand binding studies using immobilised *H. polymorpha* Pex5p and crude extracts of methanol-induced *pex1-6^{ts}* cells, showed that AO octamers did not interact with the recombinant PTS1 receptor, at conditions that allowed binding of folded GFP.SKL and dimeric DHAS. This shows that import of oligomeric proteins is not a universal pathway for peroxisomal matrix proteins.

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Keywords: *Hansenula polymorpha*; PTS1; Peroxisome

1. Introduction

Peroxisomes are ubiquitous organelles that are involved in a range of metabolic processes [1]. The enzymes that catalyse these reactions are synthesised in the cytosol and posttranslationally imported into the organelle. So far, two peroxisomal targeting signals (PTSs) have been identified: the PTS1, located at the extreme C-terminus (-SKL or variants) and the PTS2, which is found at the N-terminus of matrix proteins (consensus: -RL-X₅-H/QL). These signals are recognised by separate cytosolic receptors, Pex5p and Pex7p, respectively. After binding of their cargo, these receptors are thought to interact with a docking complex at the peroxisomal membrane. At present, several proteins involved in matrix protein import have been characterised, but still little is known of the principles of the actual translocation process [2].

In contrast to mitochondria and the endoplasmic reticulum (ER), peroxisomes can import folded and oligomeric

proteins [3,4]. Even 9-nm gold particles, coated with PTS1 peptides can be taken up by mammalian peroxisomes upon microinjection into the cells [5]. These observations are remarkable, especially because the peroxisomal membrane is impermeable for small solutes [6].

Recent other examples of folded protein import include proteins that carry a twin-arginine sorting signal and are transported across the thylakoid membrane or the bacterial cell membrane [7] and those that follow the cvt pathway for import into the vacuole (e.g. aminopeptidase I) [8].

Previous experiments in the yeast *Hansenula polymorpha* indicated that the PTS1 protein alcohol oxidase (AO) cannot be imported into peroxisomes when it is assembled into the active octameric form. Douma et al. [9] artificially introduced purified octameric AO into the cytosol of *H. polymorpha* protoplasts via liposome fusion. However, this protein remained in the cytosol under conditions that peroxisomal import was normally functional [9]. Similar results were obtained by Waterham et al. [10] who used a temperature-sensitive *pex* mutant, *pex1-6^{ts}* (formerly named *per13-6^{ts}*) of *H. polymorpha* to accumulate octameric AO in the cytosol. Upon a shift of these cells to the permissive temperature, new peroxisomes were rapidly formed, which,

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however, did not incorporate the cytosolic, octameric AO protein [10].

To study whether this is a general feature of protein import into *H. polymorpha* peroxisomes, we analysed the fate of two other folded proteins, namely a PTS1-containing green fluorescence protein (GFP.SKL) and the endogenous dimer dihydroxyacetone synthase (DHAS) in *pex1–6^{ts}*. The results presented here indicate that these proteins are normally imported after the shift of cells from restrictive to permissive growth conditions. In addition, we show that failure of octameric AO import is related to its inability to interact with the PTS1 receptor, Pex5p. As a consequence, assembly of AO into active oligomers most likely occurs upon or after import and release from the receptor, Pex5p. The crucial physiological relevance of this phenomenon is discussed.

2. Experimental procedures

2.1. Organism and growth

H. polymorpha HF27 (see below) was used in all experiments. Cells were grown in batch cultures on mineral media [11] containing (i) 0.5% (w/v) glucose, (ii) 0.5% (v/v) methanol or (iii) a mixture of 0.1% (v/v) glycerol and 0.5% methanol as carbon and energy source in the presence of 0.25% (w/v) ammonium sulfate or 0.25% (w/v) ethylamine as the nitrogen source.

Temperature shift experiments were performed as follows: cells grown at 44 °C (restrictive temperature) on glycerol/methanol/ammonium sulfate media were collected by centrifugation and incubated in glucose/ammonium sulfate media for 30 min at 44 °C to deplete methanol-induced transcripts [10]. Subsequently, the cells were collected again and resuspended in fresh glucose/ethylamine media and cultivated at 37 °C (permissive temperature).

2.2. Molecular techniques

Recombinant DNA procedures [12] and transformation of *H. polymorpha* cells [13,14] were performed as described. Expression plasmid pFEM34, containing the *eGFP.SKL* reporter gene under control of the *H. polymorpha* AO promoter (*P_{AOX}*), was constructed as follows. By PCR, using primers GFP-2 (5' AGAAAGCTTATGGT-GAGCAAGGGC 3') and KN2 (5' CCCGTCGACTTACAGCTTCGACTTGTACAGCTCGTC 3'), a *Hind*III site was introduced upstream of the *eGFP* start codon, and a SKL-encoding sequence was introduced at the extreme C-terminus of *eGFP* followed by a *Sal*I site. The 0.8-kb PCR product was digested with *Hind*III and *Sal*I and inserted in similarly digested pHIPX4 [15]. *H. polymorpha pex1–6^{ts}* [10] was transformed with pFEM34. A stable transformant, designated HF27, which contained two copies of the plasmid integrated into the promoter region of

the genomic *AOX* locus, was selected. Integration into this region does not affect expression of the endogenous *AOX* gene [13].

For isolation of C-terminally His₆-tagged *H. polymorpha* Pex5p [16], the corresponding gene was amplified by PCR, using primers PEX5HIS-1 (5' GCGCCATGG-CATTTCTGGGAGGATCGG 3') and PEX5HIS 2 (5' CGCAGATCT TATGTCGTAGGTTTTTCGG 3'). The PCR product was digested with *Nco*I and *Bgl*II and inserted into the same restriction sites of expression vector pQE 60 (Qiagen GmbH, Hilden, Germany), resulting in expression plasmid pQE-PEX5.

In *H. polymorpha*, the C-terminally His₆-tagged Pex5p is functional since it complements a *H. polymorpha* PEX5 deletion strain when synthesised under control of its own promoter (van der Klei et al., unpublished results).

2.3. Biochemical methods

Preparation of crude cell extracts [17], determination of DHAS enzyme activities [18], SDS-PAGE [19] and Western blotting [20] were performed by established procedures. Protein concentrations were determined using the Bio-Rad Protein Assay system (Bio-Rad GmbH, Munich, Germany) using bovine serum albumin as a standard.

2.4. Receptor–ligand binding assays

The *Escherichia coli* M15[pREP4] strain containing plasmid pQE-PEX5 was grown and induced as detailed in The QIAexpressionist™ (Qiagen). IPTG induction was performed at 30 °C for 3 h. Cells were collected by centrifugation and resuspended in buffer A [50 mM phosphate buffer pH 7.2, 300 mM NaCl, 10% glycerol, 1% Tween 20, 0.2 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride (PMSF) and complete protease inhibitor cocktail (EDTA free) (Roche, Almere, The Netherlands)] and subsequently broken using a French press. The homogenate was centrifuged for 20 min at 16,000 × *g* at 4 °C. The resulting supernatant was loaded onto a nickelchloride activated HiTrap (1 ml) affinity column (Amersham Pharmacia, Uppsala, Sweden). The column was washed with 2 column volumes (CV) buffer A and 3 CV of buffer B, containing 50 mM phosphate buffer pH 7.2, 100 mM NaCl, 1 mM PMSF and complete protease inhibitor cocktail (EDTA-free) at 4 °C. Subsequently, the column was washed with 2 CV of buffer B at room temperature (RT). Crude extracts of *H. polymorpha* cells were prepared in buffer B and 250 μg protein was loaded onto the HiTrap-Pex5p-His₆ column. After incubation for 2 min at RT, the flow through was collected and the column was washed with 5 CV buffer B, followed by elution of Pex5p and bound proteins with 5 CV buffer B containing 500 mM imidazole. Equal portions of the pooled flow through including wash fractions and the imidazole elution fractions were analysed by Western

blotting. In control experiments, the NiTrap column was loaded with M15[pREP4] extracts lacking Pex5p-His₆.

2.5. Microscopy

Fluorescence microscopy [17], electron microscopy and immunocytochemistry [21] were performed as described.

3. Results

3.1. *H. polymorpha* peroxisomes can import folded oligomeric proteins

Previously, Waterham et al. [10] reported that new peroxisomes, which developed in cells of a temperature-sensitive *H. polymorpha* *pex* mutant after transfer from restrictive (44 °C) to permissive growth conditions (37 °C), did not take up folded octameric AO that had accumulated in the cytosol prior to the temperature shift [10]. To analyse whether this failure was related to the model protein used in these studies, we analysed the fate of other peroxisomal proteins under similar experimental conditions.

We used a PTS1-containing green fluorescent protein (GFP) as an example of a folded, monomeric protein. The folding state of GFP can easily be monitored because only GFP protein that has obtained its correct tertiary structure shows fluorescence [22]. As oligomeric peroxisomal protein we analysed endogenous DHAS. For this protein, enzyme

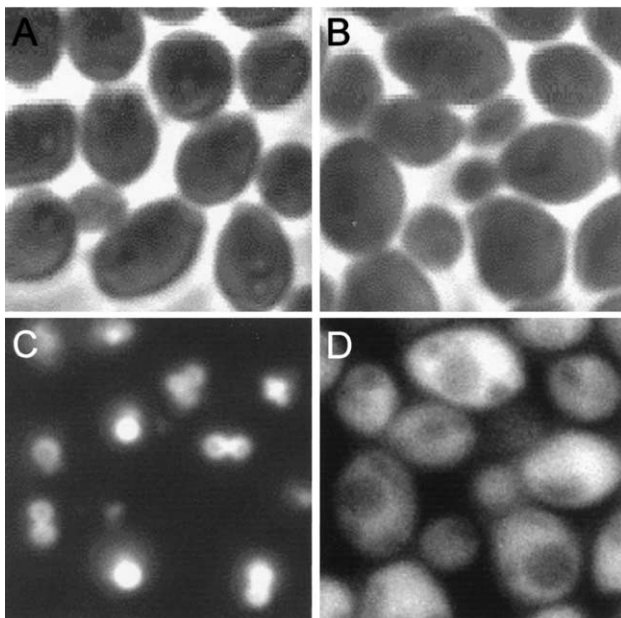


Fig. 1. GFP.SKL is peroxisomal at permissive conditions and cytosolic at restrictive conditions in *H. polymorpha* HF27. At both subcellular locations, the protein folds into the correct tertiary structure, indicated by the fluorescence. Cells were grown in methanol-containing media at permissive (37 °C; A,C) or restrictive (44 °C; B,D) conditions. (A,B) bright field images; (C,D) fluorescence images.

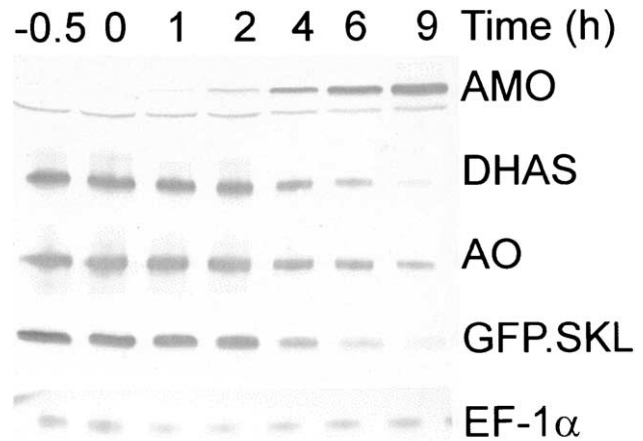


Fig. 2. Protein levels of marker proteins in *H. polymorpha* HF27 prior to ($T = -0.5$ h and $T = 0$ h) and after the shift ($T = 1-9$ h) from glycerol/methanol/ammonium sulfate at restrictive temperature (44 °C) to glucose/ethylamine media at permissive temperature (37 °C). Crude cell extracts were analysed for the levels of AO, DHAS, AMO, GFP.SKL and the constitutively produced EF-1 α (used as loading control) by Western blotting. Equal amounts of cells (OD₆₆₃ units) were loaded per lane.

activity is indicative for the folded, dimeric conformation [18].

A hybrid gene encoding GFP containing the PTS1 signal -SKL at the extreme carboxy-terminus (*GFP.SKL*) was placed under control of the AO promoter and integrated into the genome of *H. polymorpha* *pex1-6^{ts}*. The resulting strain, designated HF27, was grown under conditions that strongly induce AO, DHAS and *GFP.SKL* synthesis at both the permissive and restrictive temperature. As expected, all three proteins were located inside peroxisomes in cells grown at the permissive temperature but mislocated to the cytosol of restrictive cells (Fig. 1, GFP; AO and DHAS—not shown, see Ref. [10]).

Fluorescence microscopy indicated that the cells showed GFP-fluorescence independent of the growth temperature. Hence, the protein normally folds both in the cytosol and in the peroxisomal matrix (Fig. 1). Enzyme activity measurements revealed that DHAS activities were at WT levels at both growth temperatures (values ranging from 0.10 to 0.16 U/mg protein), demonstrating that both in peroxisomes and in the cytosol, this enzyme was properly folded and oligomerised. In line with earlier reports also AO assembly/activation occurred at both subcellular locations and temperatures (data not shown, see Ref. [10,23]).

Subsequently, we used strain HF27 in a shift experiment essentially as described previously [10]. HF27 cells, induced in methanol/ammonium sulfate-containing media at the restrictive temperature, were shifted to glucose/ethylamine-containing media at permissive conditions. In these cultures, the AO production level is below the limit that induced AO crystallization in the cytosol [10]. Upon the shift, the synthesis of AO, *GFP.SKL* and DHAS is fully repressed by glucose, whereas the synthesis of a new peroxisomal enzyme, the PTS2 protein amine oxidase (AMO), is induced (Fig. 2,

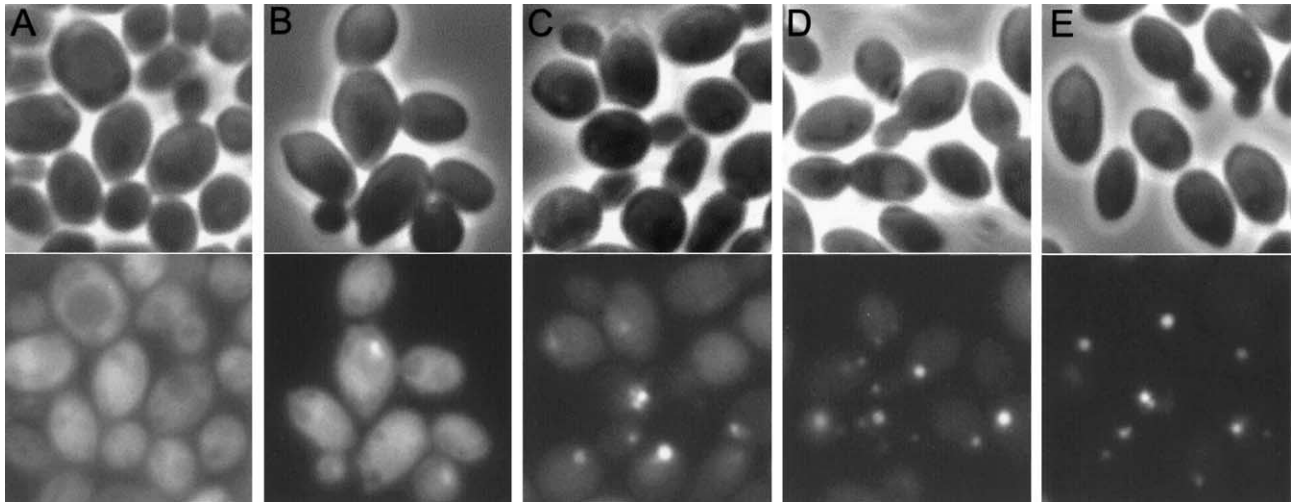


Fig. 3. Cytosolic GFP.SKL is sorted to peroxisomes in HF27 cells shifted from restrictive to permissive conditions. The subcellular location of GFP.SKL was determined by fluorescence microscopy. Top panels, bright field images; bottom panels, fluorescence images. (A) Prior to shift, (B) 1 h, (C) 2 h, (D) 4 h and (E) 6 h after the shift to glucose/ethylamine-media and permissive temperature (37 °C).

see also Ref. [10] [13]. At the same time, peroxisomes are formed again due to lowering the temperature to permissive conditions.

Fluorescence microscopy revealed that upon the shift, the diffuse GFP fluorescence gradually changed into distinct fluorescent dots. The relative intensity of these dots gradu-

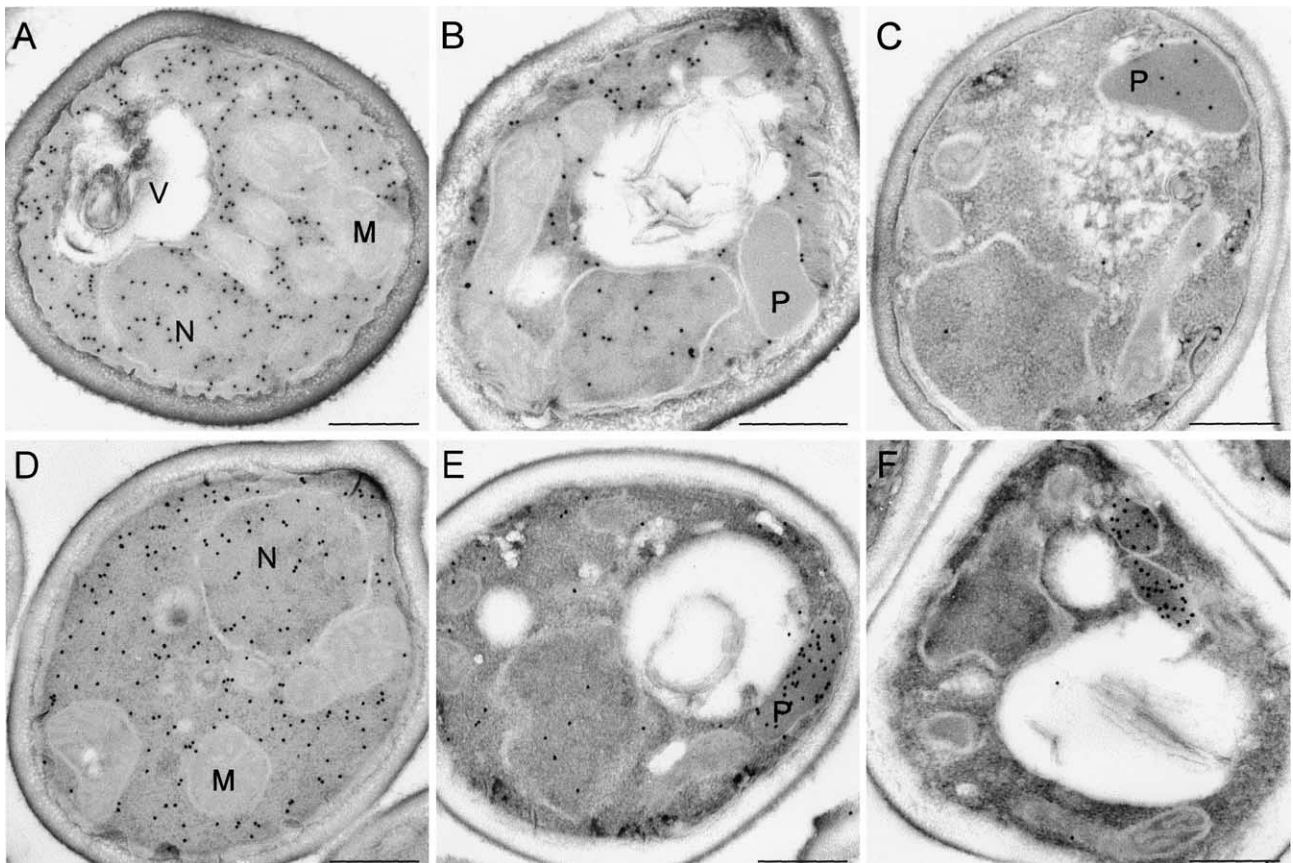


Fig. 4. Differential import of PTS1 proteins in HF27 cells shifted to glucose/ethylamine media and permissive temperature. Immunocytochemical detection of AO (A,B), GFP.SKL (C) and DHAS (D–F) in *H. polymorpha* HF27 cells grown on glycerol/methanol at restrictive conditions (44 °C; A,D) and 4 h (C,E) or 8 h (B,F) after a shift to glucose/ethylamine-medium at permissive conditions (37 °C). Abbreviations: M—mitochondrion, N—nucleus, P—peroxisome, V—vacuole. Bar=0.5 µm.

ally increased during further cultivation concomitant with a gradual decrease of the cytosolic fluorescence, which became undetectable 6 h after the shift (Fig. 3). Electron microscopic analysis revealed that the fluorescent dots represented GFP-containing peroxisomes (Fig. 4C). Immunocytochemical experiments revealed that also cytosolic DHAS (Fig. 4D)—but not AO (Fig. 4A,B)—became imported into the organelles that developed after the shift (Fig. 4E,F). Like GFP.SKL, the location of DHAS gradually changed from cytosolic (Fig. 4D) to peroxisomal within 8 h after the shift to permissive conditions (Fig. 4E,F).

3.2. Folded DHAS and GFP.SKL, but not AO, can bind to Pex5p

A likely explanation for the failure of assembled AO to become imported into peroxisomes is that the octameric protein is unable to bind to the PTS1 receptor, Pex5p. To check this possibility, we analysed binding of AO, GFP.SKL and DHAS protein to Pex5p in vitro, using recombinant His₆-tagged Pex5p immobilised on a nickel column and cell-free extracts prepared from methanol-induced HF27 cells grown at restrictive temperature. As shown in Fig. 5, DHAS and GFP.SKL were retained on this column, indicative for a specific interaction with Pex5p. In contrast, AO protein was detected only in the flow-through fractions, suggesting that the AO octamers present in the extract could not bind to Pex5p. Sucrose gradients of crude extracts of cells grown at restrictive and permissive temperatures showed that at both temperatures the level of monomeric

AO protein is comparable and very low relative to octameric AO (data not shown). Also, after Western blotting of equal amounts of AO enzyme activity (50 mUnits), the AO protein bands were comparable. Taken together, these data indicate that at both temperatures, AO protein is properly folded and normally active. Hence, the failure of AO to bind to Pex5p cannot be due to improper folding or aggregation of the AO protein synthesised at restrictive temperatures.

4. Discussion

Peroxisomes can import large folded proteins (reviewed in Ref. [24]). In this study, we demonstrate that peroxisomes of *H. polymorpha* are also capable to do so since folded GFP.SKL and dimeric DHAS could be imported into these organelles. However, octameric AO remained in the cytosol. These data are in line with earlier findings, which indicated that octameric AO is not imported into peroxisomes of *H. polymorpha* [9,10]. The lack of AO import is not due to the formation of AO aggregates/crystals. AO octamer crystal formation is prevented by controlling the AO protein production level during growth [10], while AO aggregate formation, a phenomenon that is only observed in case of AO monomers, is not likely since AO monomer concentration is extremely low, comparable to WT situations. Obviously, an intrinsic feature of this protein is interfering.

An early step in PTS1 protein import is binding of the targeting signal to the receptor, Pex5p. We show that octameric AO has lost the capacity to interact with Pex5p, whereas folded GFP.SKL and enzymatically active (and thus dimeric) DHAS still could bind to the receptor. Probably, the PTS of AO became buried upon folding/oligomerisation. This implies that under normal conditions in wild-type cells, AO assembly occurs at a relatively late stage in the import process after interaction of the PTS with Pex5p. This is in line with other data that suggested that AO oligomerises in the peroxisomal matrix [25] and stresses the apparent capacity of *H. polymorpha* peroxisomes to assemble matrix proteins. This mode of assembly/activation is of major physiological importance: *H. polymorpha* cells must have invented a way to prevent preliminary activation of AO in the cytosol since only minor amounts of enzymatically active AO in this compartment prevent growth of the cells on methanol [23].

Our current data reveal that DHAS can be imported as an oligomer in *H. polymorpha*. Earlier studies by Sakai et al. [26] with *C. boidinii* suggested that DHAS was taken up as monomers, which subsequently assembled in the matrix. Very recently, Stewart et al. [27] demonstrated in an elegant series of pulse chase experiments that *C. boidinii* DHAS assembles in the cytosol before import. However, as in *H. polymorpha*, also in *C. boidinii* AO protein is assembled after import [27].

So far, there is one clearcut example of an endogenous peroxisomal protein that requires oligomerisation prior to

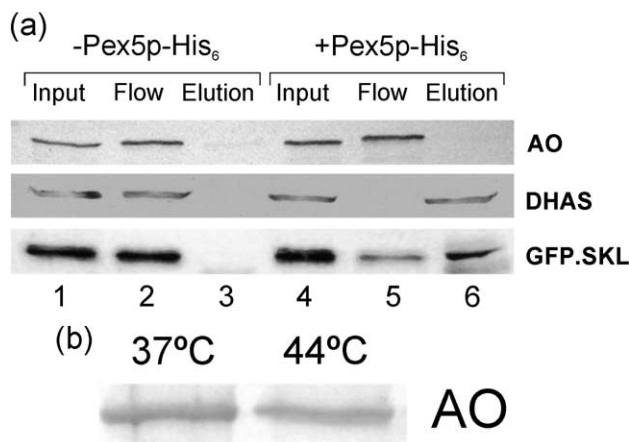


Fig. 5. (a) In vitro binding of folded, assembled PTS1 proteins with recombinant Pex5p. A crude extract of *H. polymorpha* HF27 cells grown in glycerol/methanol media at restrictive temperature (44 °C) was loaded onto an Ni-column preloaded with *E. coli* extract without Pex5p-His₆ (lanes 1–3) or with Pex5p-His₆ (lanes 4–6). Equal portions of crude extracts (input, lanes 1 and 4), flow-through, wash (flow, lanes 2 and 5) and elution (lanes 3 and 6) were analysed by Western blotting using antibodies against AO, DHAS and GFP. (b) Western blot of equal amounts of specific AO activity (5 m Units) that were present in crude extracts of HF27 cells, grown in glycerol/methanol-media at permissive (37 °C) and restrictive (44 °C) temperatures. The density of the AO protein bands is comparable, indicating that AO protein is normally active at permissive temperature.

import. In *Yarrowia lipolytica*, cytosolic assembly of thiolase into dimers, a process that requires Pex20p, is necessary for import [28].

Taken together, the picture that emerges from our and the other data is that depending on the protein examined specifically only monomers (*H. polymorpha* AO), only oligomers (*Y. lipolytica* thiolase) or either of the two forms of a protein (DHAS) can be imported into peroxisomes.

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References

- [1] H.F. Tabak, I. Braakman, B. Distel, Trends Cell Biol. 9 (1999) 447–453.
- [2] E.H. Hettema, B. Distel, H.F. Tabak, Biochim. Biophys. Acta 1451 (1999) 17–34.
- [3] J.R. Glover, D.W. Andrews, R.A. Rachubinski, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 10541–10545.
- [4] J.A. McNew, J.M. Goodman, J. Cell Biol. 127 (1994) 1245–1257.
- [5] P.A. Walton, S.J. Gould, J.R. Feramisco, S. Subramani, Mol. Cell. Biol. 12 (1992) 531–541.
- [6] Y. Elgersma, H.F. Tabak, Biochim. Biophys. Acta 1286 (1996) 269–283.
- [7] A. Mant, I. Karnauchov, S. Brink, R.G. Herrmann, R.B. Klossgen, C. Robinson, EMBO J. 14 (1995) 2715–2722.
- [8] J. Kim, D.J. Klionsky, Annu. Rev. Biochem. 69 (2000) 303–342.
- [9] A.C. Douma, M. Veenhuis, A.J.M. Driessen, W. Harder, Yeast 6 (1990) 99–105.
- [10] H.R. Waterham, V.I. Titorenko, G.J. Swaving, W. Harder, M. Veenhuis, EMBO J. 12 (1993) 4785–4794.
- [11] J.P. van Dijken, R. Otto, W. Harder, Arch. Microbiol. 111 (1976) 137–144.
- [12] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
- [13] K.N. Faber, P. Haima, C. Gietl, W. Harder, G. Ab, M. Veenhuis, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 12985–12989.
- [14] K.N. Faber, P. Haima, W. Harder, M. Veenhuis, G. Ab, Curr. Genet. 25 (1994) 305–310.
- [15] C. Gietl, K.N. Faber, I.J. van der Klei, M. Veenhuis, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 3151–3155.
- [16] I.J. van der Klei, R.E. Hilbrands, G.J. Swaving, H.R. Waterham, E.G. Vrieling, V.I. Titorenko, J.M. Cregg, W. Harder, M. Veenhuis, J. Biol. Chem. 270 (1995) 17229–17236.
- [17] R.J.S. Baerends, K.N. Faber, A.M. Kram, J.A.K.W. Kiel, I.J. van der Klei, M. Veenhuis, J. Biol. Chem. 275 (2000) 9986–9995.
- [18] L.V. Bystrykh, W. de Koning, W. Harder, Methods Enzymol. 188 (1990) 435–445.
- [19] U.K. Laemmli, Nature 227 (1970) 680–685.
- [20] J. Kyhse-Andersen, J. Biochem. Biophys. Methods 10 (1984) 203–209.
- [21] H.R. Waterham, V.I. Titorenko, P. Haima, J.M. Cregg, W. Harder, M. Veenhuis, J. Cell Biol. 127 (1994) 737–749.
- [22] R.Y. Tsien, Annu. Rev. Biochem. 67 (1998) 509–544.
- [23] I.J. van der Klei, W. Harder, M. Veenhuis, Arch. Microbiol. 156 (1991) 15–23.
- [24] S. Subramani, Physiol. Rev. 78 (1998) 171–188.
- [25] M.E. Evers, W. Harder, M. Veenhuis, FEBS Lett. 368 (1995) 293–296.
- [26] Y. Sakai, A. Saiganji, H. Yurimoto, K. Takabe, H. Saiki, N. Kato, J. Cell Biol. 134 (1996) 37–51.
- [27] M.Q. Stewart, R.O. Esposito, J. Gowani, J.M. Goodman, J. Cell Sci. 114 (2001) 2863–2868.
- [28] V.I. Titorenko, J.J. Smith, R.K. Szilard, R.A. Rachubinski, J. Cell Biol. 142 (1998) 403–420.